Synthesis of 6-Hydroxy δ-Lactones and 5,6-Dihydroxy Eicosanoic/Docosanoic Acids from Meadowfoam Fatty Acids *via* a Lipase-Mediated Self-Epoxidation

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ABSTRACT: Meadowfoam fatty acids were reacted with hydrogen peroxide in a lipase-catalyzed autocatalytic system, forming a mixture of 5,6-epoxyeicosanoic, 13,14-epoxydocosanoic, 5,6-epoxydocosanoic, and 5,6-13,14-diepoxydocosanoic acids in 98% yield. The 5,6-epoxy acids were cyclized to 6-hydroxy δ-eicosanoic/docosanoic lactones by sulfuric acid catalysis in high yield (99%). 5,6-Dihydroxy acids could be obtained from 6-hydroxy δ-lactones by a simple alkaline work-up procedure. Meadowfoam fatty acids were converted (77% yield) in a one-pot reaction to 6-hydroxy δ-lactones by *in situ* performic acid epoxidation and subsequent addition of sulfuric acid.

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KEY WORDS: 5,6-13,14-Diepoxydocosanoic acid, dihydroxy eicosanoic acid, 5,13-docosadienoic acid, δ-eicosanolactone, 5-eicosenoic acid, epoxidation, 5,6-epoxyeicosanoic acid, formic acid, hydrogen peroxide, 6-hydroxy-δ-lactones, lactonization, lipase, performic acid, sulfuric acid.

Limnanthes alba or meadowfoam is a crop currently cultivated in the Willamette valley of Oregon. Meadowfoam produces a seed oil with novel properties, which are related to its high content (64%) of the unique 5-eicosenoic acid. The remaining fatty acids are 5,13-docosadienoic acid (19%), 5-docosenoic acid (3%), and 13-docosenoic acid (10%). Unsaturation in the 5-position comprises 90% of the fatty acids present. Utilization of this double bond in close proximity to the carboxyl group has been successful for synthesizing estolides and δ-lactones by a mineral acid-catalyzed process (1–3). In addition to δ-lactones, 6-hydroxy δ-lactones have been synthesized by Fore and Sumrell (4) from epoxidized meadowfoam fatty acids. We now report an improved synthesis of epoxy fatty acids and of 6-hydroxy δ-lactones. We also investigated factors that affect ring closure to lactones. δ-Eicosanoic/docosanoic lactones are versatile precursors to amides, esters, and ethers *via* several synthetic routes (5; Isbell, T.A., and B.A. Plattner, submitted for publication).

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EXPERIMENTAL PROCEDURES

Materials. Meadowfoam fatty acids were obtained by hydrolysis of meadowfoam oil supplied by The Fanning Corp. (Chicago, IL). Ethyl acetate, acetone, and hexane were obtained from Fisher Scientific Co. (Fairlawn, NJ). Methylene chloride, toluene, acetonitrile, and monobasic phosphate were obtained from EM Science (Gibbstown, NJ). Sulfuric acid was obtained from Chemical General (Pittsburg, CA). *p*-Toluene sulfonic acid was obtained from Matheson & Coleman & Bell (Norwood, OH). Lipase sp 435 was obtained from Novo-Nordisk Inc. (Danbury, CT). Filter paper was obtained from Whatman (Maidstone, England).

Instrumentation. High-performance liquid chromatography (HPLC) analysis of fatty acid mixtures was carried out with a Thermo Separations (Fremont, CA) spectra system AS1000 autosampler/injector with a P2000 binary gradient pump coupled to a Varex ELSD III light-scattering detector (Alltech Associates, Deerfield, IL). A Dynamax silica (25 cm × 4.6 mm, 60Å, 8 μm) column from Rainin Instrument Co. (Woburn, MA) was used to separate the epoxides and lactones upon eluting with an isocratic hexane/acetone (70:30) solvent system at 1 mL/min. Retention times for eluted peaks: 3.6 min for the unsaturated fatty acids, 4.2 min for eicosanoic acid epoxide, 5.9 min for 6-hydroxy-δ-eicosanolactone and 8.0 min for 6-hydroxy-13,14-epoxy-δ-docosanolactone.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker ARX-400 with a 5-mm dual proton/carbon probe and $CDCl_3$ as solvent.

^{1}H NMR of 5,6-epoxy eicosanoic acid: δ 2.91–2.88 [*m*, 2H, –C*H*(O)–C*H*–], 2.40–2.30 (*m*, 2H, =CH–CH_2–CH$_2$–), 1.62–1.23 (*m*, 30H, –CH_2–), and 0.88–0.84 ppm (*t*, 3H, –CH_3).

^{13}C NMR of 5,6-epoxy eicosanoic acid: δ 178 (*s*), 57.3 (*d*), 57.3 (*d*) , 34.0 (*t*), 31.8 (*t*), 31.8 (*t*), 29.6 (*t*), 29.6 (*t*), 29.5 (*t*), 29.4 (*t*), 29.4 (*t*), 29.4 (*t*), 29.3 (*t*), 29.1 (*t*), 29.0 (*t*), 27.7 (*t*), 26.5 (*t*), 24.6 (*t*), 22.6 (*t*) and 14.0 ppm (*q*).

^{1}H NMR of 6-hydroxy-δ-eicosanolactone: δ 4.16–4.14 [*m*, 1H, –CH(OH)–C*H* (O)–CH$_2$–], 3.57–3.48 [*m*, 1H, –CH$_2$–C*H*(OH)–CH(O)–], 2.62–2.22 (*m*, 3H), 1.86–1.17 (*m*, 29H), and 0.85 ppm (*t*, *J* = 6.8 Hz, 3H).

^{13}C NMR of 6-hydroxy-δ-eicosanolactone: δ 171.4 (*s*), 83.2 (*d*), 73.3 (*d*), 32.6 (*t*), 31.8 (*t*), 29.6 (*t*), 29.6 (*t*), 29.6 (*t*), 29.6 (*t*), 29.6 (*t*), 29.5 (*t*), 29.5 (*t*), 29.5 (*t*), 29.5 (*t*), 29.3 (*t*), 25.4 (*t*), 24.1 (*t*), 22.6 (*t*), 18.4 (*t*), and 14.0 ppm (*q*).

^{1}H NMR of 6-hydroxy-13,14-epoxy-δ-docosanolactone: δ 4.16–4.13 [*m*, 1H, –CH(OH)–C*H*(O)–CH_2–], 3.58–3.50 [*m*, 1H, –CH_2–C*H*(OH)–CH(O)–], 2.89–2.82 [*m*, 2H, –C*H*(O)–C*H*–], 2.60–2.52 (*m*, 1H), 2.45–2.25 (*m*, 2H), 1.97–1.19 (*m*, 31H), and 0.83 ppm (*t*, J = 6.7, 3H).

^{13}C NMR of 6-hydroxy-13,14-epoxy-δ-docosanolactone: δ 171.7 (*s*), 83.2 (*d*), 73.1 (*d*), 57.3 (*d*), 57.3 (*d*), 32.5 (*t*), 31.8 (*t*), 31.7 (*t*), 29.6 (*t*), 29.6 (*t*), 29.5 (*t*), 29.4 (*t*), 29.4 (*t*), 29.4 (*t*), 29.4 (*t*), 29.3 (*t*), 29.1 (*t*), 27.7 (*t*), 27.7 (*t*), 26.5 (*t*), 25.3 (*t*), 24.0 (*t*), 22.6 (*t*), 18.3 (*t*), and 14.0 ppm (*q*).

METHODS

Lipase-catalyzed epoxidation reactions. Meadowfoam fatty acids (5 g, 16 mmol) were dissolved in 10 mL toluene or methylene chloride and warmed to 20–45°C. Lipase sp 435 (5% w/w) [immobilized on polyacrylate resin, (0.25 g, totally)], derived from *Candida antarctica,* was added. 1.5 Molar equivalents of 30% wt/vol hydrogen peroxide (2.75 mL, 24 mmol) were added in four equal portions over 6 h. Mixing of the components was maintained by overhead stirring throughout the course of the reaction. The product distribution was monitored by normal-phase HPLC (conditions described above). The epoxide product was worked up by filtering to recover the enzyme, which was kept under toluene for later reuse. The organic phase was washed with water and dried over $MgSO_4$, and the solvent was removed *in vacuo* to yield the desired products.

6-Hydroxy-δ-lactone synthesis. The epoxide of meadowfoam fatty acids (1 g, 3.07 mmol) from above was dissolved in 2–10 mL organic solvent (hexane, toluene, methylene chloride, acetonitrile, or ethyl acetate) and mixed by magnetic stirrer. Sulfuric acid (5 µL, 9 µmol) was added, and the reaction was kept at room temperature for 24 h. The organic phase was washed with 10 mL water, and 5 mL toluene was added and distilled to remove any remaining water as an azeotrope under reduced pressure, to yield the desired products.

Formic acid-catalyzed epoxidation of meadowfoam fatty acids and consecutive formation of 6-hydroxy-δ-lactone epoxides in one pot. Meadowfoam fatty acids (5 g, 16 mmol) were dissolved in 10 mL methylene chloride or toluene. Formic acid (0.61 g) was added, the reaction was cooled to 5°C, and then 30% wt/vol hydrogen peroxide (3.67 mL, 32 mmol) was added slowly over 1 h. Mixing of the components was maintained by overhead stirring throughout the course of the reaction. The product distribution was monitored by HPLC. After 80 h, either *p*-toluene sulfonic acid (15 mg, 80 µmol) or sulfuric acid (44 µL, 80 µmol) was added. After 12 h, the organic phase was washed with water or a weak base to remove the acid. Then, the remaining water and solvent were removed through azeotropic distillation with toluene to yield the desired product.

RESULTS AND DISCUSSION

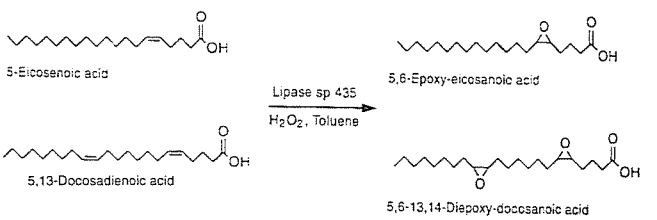
Meadowfoam fatty acids contain 90% Δ5 and Δ5,13 unsaturated C_{20} and C_{22} fatty acids, with the remaining portion being erucic acid. The literature is abundant on the subject of double-bond epoxidation. However, until now there has been no one process superior in yield, safety, and cost. Epoxidation of meadowfoam fatty acids has been reported (4) with a peracetic/acetic acid system free of sulfuric acid, which provided a 50% yield of epoxide. However, due to the low yield and the prewash of peracetic acid to remove the sulfuric acid, this method is not practical on an industrial scale. In light of these results, we have investigated a lipase-mediated epoxidation with lipase sp 435 in toluene or methylene chloride that employs a slow addition of hydrogen peroxide (6,7). This method yielded a high-purity epoxide product that contained little unreacted material or diol by-product (Table 1 and Scheme 1). The reaction product also contains varying amounts of a mixture of 6-hydroxy-δ-lactones, depending on the solvent used. The discovery of a δ-lactone-containing material within the reaction mixture prompted us to investigate the possible conversion of the epoxy fatty acids into 6-hydroxy δ-lactones. Previous lactonization studies in our lab (3) indicated that the development of a carbocation intermediate at the Δ5 position is stabilized by the proximal carboxylic functionality. Epoxides are known to be sensitive to acid catalysts and undergo cationic polymerizations in the presence of strong acid catalysts. We envisioned that coupling these two chemical properties within the 5,6-epoxy fatty acids would result in their facile lactonization.

Table 2 reports the effects of sulfuric acid concentration on the distribution of lactone vs. the intermolecular condensation by-product, such as hydroxy estolides (Scheme 2). As acid concentration increases, formation of the estolides also

TABLE 1
Epoxidation of Meadowfoam Fatty Acids by Different Epoxidation Techniques

Catalyst	Solvent	% Epoxide in product[a]	% Lactone in product[a]	% Diol in product[a]
Formic acid	CH_2Cl_2	37	56	0
Lipase sp 435[b]	Toluene	80	12	3
Lipase sp 435[b]	CH_2Cl_2	98	1	0

[a]Yield determined by high-performance liquid chromatography, Dynamax silica 25 cm × 4.6 mm i.d. column Rainin Instrument Co. (Woburn, MA). Hexane/acetone 70:30, 1 mL/min.
[b]Novo-Nordisk Inc. (Danbury, CT).

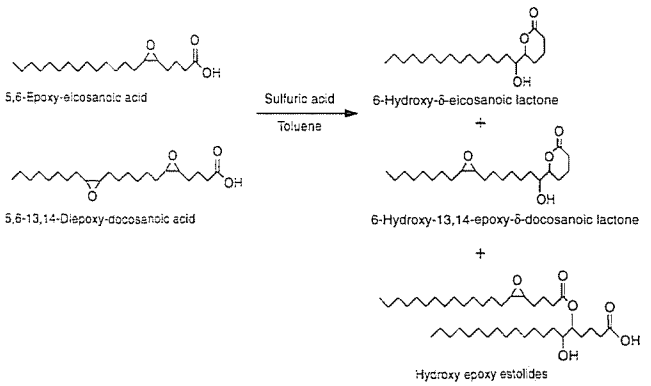


SCHEME 1

TABLE 2
Lactonization of Neat Epoxidized Meadowfoam Fatty Acids with Sulfuric Acid at 60°C

Mole% sulfuric acid	Time (h)	% Lactone[a]	% Estolide[a]
1	2	58	2
3	2	47	38
10	2	56	24
1	12	88	2
3	12	36	48
10	12	7	69

[a]Yield determined by high-performance liquid chromatography with a Dynamax silica 25 cm × 4.6 mm i.d. column; hexane/acetone 70:30, 1 mL/min. See Table 1 for company source.



SCHEME 2

TABLE 3
Lactonization of Epoxidized Meadowfoam Fatty Acids with Sulfuric Acid[a]

Solvent	Solvent/g (mL)	Sulfuric acid (mole %)	Lactone (%)[b]	Estolide (%)[b]
Hexane	5	1	14	—
Hexane	5	3	22	3
Hexane	5	10	80	14
Hexane	10	1	23	1
Hexane	10	3	38	2
Hexane	10	10	55	8
Ethyl acetate	5	1	84	—
Ethyl acetate	5	3	89	2
Ethyl acetate	5	10	67	3
Ethyl acetate	10	1	71	—
Ethyl acetate	10	3	95	—
Ethyl acetate	10	10	99	1
Toluene	5	1	58	—
Toluene	5	3	96	—
Toluene	5	10	77	17
Toluene	10	1	60	3
Toluene	10	3	85	3
Toluene	10	10	99	—
Acetonitrile	5	3	67	4
Acetonitrile	5	10	96	3
Acetonitrile	10	1	57	6
Acetonitrile	10	3	82	5
Acetonitrile	10	10	96	4

[a]Reaction time 2 h at room temperature.
[b]Yield as determined by high-performance liquid chromatography, Dynamax silica 25 cm × 4.6 mm i.d. column; hexane/acetone 70:30, 1 mL/min. See Table 1 for company source.

increases. The optimal lactone formation conditions occur at low acid concentration (1 mole% H_2SO_4) and longer reaction times (12 h). However, after 12 h, the yield of the desired lactone drastically decreases (Fig. 1) in favor of estolide formation (Scheme 2).

The intramolecular process, lactonization, can be promoted by dilution of the epoxide in an appropriate solvent, which will help promote the polarization of the epoxide bond. Tables 3 and 4 report the positive role of a dipolar solvent on the formation of lactone. In the absence of solvent, or in a nonpolar solvent such as hexane, low yields of lactone are obtained. However, the effect of dilution, from neat to hexane medium, on the intermolecular estolide condensation pathway was observed as a 3-fold decrease in the formation of estolide (Table 4). Of particular interest is the lack of correlation between increasing dipole moment of the solvent and the formation of lactone once a minimal value of solvent polarity was achieved. Those solvents with dipole moments equal to or greater than toluene promote lactonization readily under these conditions, with virtual exclusion of the estolide.

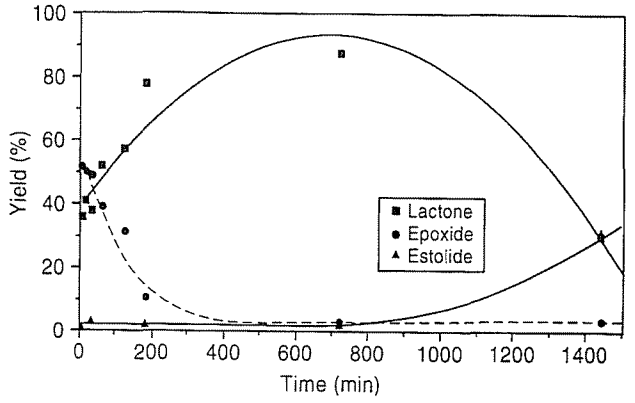


FIG. 1. Lactone formation when epoxidized meadowfoam fatty acids are reacted neat with 1 mole% sulfuric acid at 60°C.

TABLE 4
Solvent Dipole Effect on Lactone Yield

Solvent	Dipole (debeye)[a]	mL (g)	H_2SO_4 (mole, %)	Lactone[b]	Estolide[b]
None	—	—	10	56	24
Hexane	0	10	10	55	8
Toluene	0.36	10	10	99	—
CH_2Cl_2	1.6	10	10	93	—
EtOAc	1.78	10	10	99	1
CH_3CN	3.92	10	10	96	4

[a]Dipole of gas-phase molecule.
[b]Yield determined by high-performance liquid chromatography, Dynamax silica 25 cm × 4.6 mm i.d. column; hexane/acetone 70:30, 1 mL/min. See Table 1 for company source.

TABLE 5
Lactonization of Epoxidized Meadowfoam Fatty Acids with Sulfuric Acid in Methylene Chloride: Effect of Solvent Concentration[a]

Methylene chloride (mL)/acids (g)	Sulfuric acid (mmolal)	Lactone[b]	Estolide[b]
2	46	75	7
2	153	69	17
2	1530	54	23
2	4600	32	41
5	6	56	5
5	18	81	9
5	61	89	9
5	610	66	16
5	1840	55	34
10	3	57	—
10	9	86	—
10	31	93	—
10	310	93	—
10	920	88	12

[a]Reaction time 2 h at room temperature.
[b]Yield as determined by high-performance liquid chromatography, Dynamax silica 25 cm × 4.6 mm i.d. column; hexane/acetone 70:30, 1mL/min. See Table 1 for company source.

To further explore the role of solvent and acid on the lactonization of 5,6-epoxy acids, a study was performed on the role of methylene chloride with respect to solvent and acid concentration. These results are shown in Table 5. The amount of solvent present does have some limited lactonization-promoting effects (>5 mL CH_2Cl_2/g acids). The optimal sulfuric acid concentration is between 30–60 mmolal with no increased yields of δ-lactone at higher acid concentrations.

To demonstrate how the 6-hydroxy-δ-lactone could be produced in one pot, we utilized the industrial process of generating performic acid *in situ* to epoxidize meadowfoam fatty acids. Sulfuric or *p*-toluene sulfonic acid was added consecutively to yield 77% 6-hydroxy-δ-lactones.

In summary, the lipase-mediated oxidation of meadowfoam fatty acids produces the highest-purity epoxy products, thus providing superior starting material for the subsequent synthesis of 6-hydroxy δ-lactones. The synthesis of hydroxy δ-lactones is achieved best by the presence of low concentrations of sulfuric acid.

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